REMARKS

The invention process essentially entails five steps.

They are:

(a) growing a culture of cells of algae microorganism photoheterotrophically in a Tris-acetate-phosphate medium under white fluorescence illumination conditions to accumulate an endogenous substrate;

(b) depleting a nutrient selected from the group consisting of sulfur, iron, and/or manganese from the medium in the presence of DCMU[1] by suspending said culture of cells in the absence of said nutrient and sealing the culture of cells of algae microorganism from atmospheric oxygen until conditions become anaerobic;

(c) measuring the rate of cellular oxidative respiration in m mol O_2 (mol Chl) $^{-1}s^{-1}$ of a sample of cells of said suspended of algae microorganism from step (b) in the dark until it is constant or about 13 m mol O_2 (mol chl)$^{-1}s^{-1}$;

(d) measuring the rate of O_2 evolution of a sample of the algae microorganism from step (c) under light of saturating intensity of yellow actinic excitation at about 1,300 μm photons $m^{-2}s^{-2}$;

(e) inducing reversible hydrogenase through photosynthesis by controlling the light saturated rate of oxygen production from the culture of cells of algae microorganism of step (b) so that it is equal to or less than a the constant or 13 μ mol O_2 (mol Chl)-$^{1}s^{-1}$ rate of cellular

[1] 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU)
See Plant Biology Vol. 95, Issue 15, 9009-9013, July 21, 1998.

oxidative respiration using saturating blue actinic excitation at 250 μ mol photons $m^{-2}s^{-1}$ at 700 nm to generate an evolved gas that includes hydrogen.

The invention process continuously produces hydrogen by inducing reversible hydrogenase in a manner that provides activity of photosynthesis from a light saturated rate of oxygen production (P) equal to or less than the rate of cellular respiration of algae microorganism (as shown by the R curve in FIG. 1).

It is well known in the prior art for about 60 years that algae will not produce hydrogen gas when oxygen is present because the hydrogenase enzyme that releases hydrogen is not synthesized and is not stable when oxygen is present, wherein the normal plant/algae photosynthetic process splits water and produces oxygen as a by-product, and wherein to get algae to induce the hydrogenase enzyme it has been necessary to use physical (i.e., inert gas bubbling) or chemical (addition of strong reducing agents or biochemical, oxygen-scrubbing systems) means to get rid of the oxygen.

The invention process resolves this problem by its discovery of a metabolic switch, whereby removing sulfate from the medium of healthy growing algae rapidly decreases the innate ability of the algae to split water and produce oxygen to only about 10% of their normal ability over a 15 to 30 hour period of time. In this sulfate removal process, applicants have further discovered that algae respiration can take up oxygen at about the level or a little greater (rate) than the cells can produce oxygen (at the lower level of production ability) under sulfur-deprived conditions, and the culture under these conditions will metabolize all the remaining oxygen in a sealed-off culture medium, whereupon the system will rapidly become anaerobic. Hydrogen production continues under these conditions because the hydrogenase enzyme induced in the

cells under illumination is stable under controlled oxygen conditions while cell respiration necessary for photosynthesis continues.

By contrast, Greenbaum '211 only discloses producing H_2 and O_2 by use of algae in light comprising:

1) subjecting algae in an aqueous phase to light in an environment free of CO_2 and atmospheric O_2 to produce H_2 and O_2 by the action of the light-stimulated algae in splitting water molecules during a first period of time of sufficient duration to produce a physiological stress on said algae;

2) culturing the algae in culture medium in an aerobic atmosphere during a second period of time sufficient to remove the physiological stress; and

3) subjecting the algae in an aqueous phase to light in an environment free of CO_2 and atmospheric O_2 during a third period of time at an enhanced rate of production of H_2 and O_2 compared to that occurring during the first time period of step 1).

Greenbaum '211 lacks applicants' step (b) of depleting sulfur in the presence of DCMU and sealing off the culture cells until the culture becomes substantially anaerobic.

Greenbaum '211 further lacks applicants' steps (c),(d) and (e) which require measuring cellular respiration in the dark, incubating in light of saturating intensity of yellow actinic excitation and measuring the light-saturated rate of O_2 evolution, and inducing reversible hydrogenase through photosynthesis by controlling the light saturated rate of oxygen production so that it is equal to or less than the rate of cellular respiration.

Weetall '076 disclose continuous photometabolic production of a useful product, comprising immobilizing whole cells of a photometabolically active organism on a medium to form a stabilized composite, placing the composite within a reactor having at least one light

transmitting wall, and, in the presence of light being transmitted through the wall, continuously passing into the reactor a substance capable of being photometabolized by the cells under conditions sufficient to assure the production of a useful product.

Although blue-green algae may be used in the biophotolysis of water by oxidizing the water and reducing NADP to NADPH, it is clear that the combination of Weetall '076 with Greenbaum '211 fails to suggest or teach applicants' steps (b), (c), (d) and (e). Depletion of the sulfur nutrient and incubating the culture in light to induce reversible hydrogenase to provide activity of photosynthesis from a light saturated rate of oxygen production, equal to or less than the rate of cellular respiration cannot even by remotely inferred from the combination.

The deficiencies of the combination of Greenbaum '211 and Weetall '076 are not compensated for by any teachings in the secondary reference of Wykoff et al.

Wykoff et al. disclose the extent to which the light-saturated rate of photosynthetic O_2 evolution declines in *Chlamydomonas reinhardtii* upon P and S starvation. It makes no reference to or acknowledgement of, the prior art problem of not being able to sustain hydrogen production due to deactivation of hydrogenase in the presence of oxygen during photosynthetic hydrogen production. Neither does Wykoff et al. provide any solution to this problem.

Thus, even if Wykoff et al. were combined with Greenbaum '211 and Wetall '076, applicants' process claims as presently amended could not possibly result from this combination.

Neither would applicants' process be obvious because no reference alone or in combination either acknowledge or resolve the problem of providing sustained production of hydrogen by avoiding deactivation of hydrogenase in presence of oxygen by controlling the light saturated rate of oxygen production so that it is equal to or less than the rate of cellular respiration.

Even if the Wykoff et al. teachings of the extent to which the light saturated rate of photosynthetic O_2 evolution declines in *Chlamydomonas reinhardtii* upon P and S starvation were substituted into the processes of the primary references of Greenbaum '211 and Wetall '076, the substitution would be inadequate without hindsight reference to applicants' invention to provide a skilled person in the art with means for sustaining production of hydrogen by avoiding deactivation of hydrogenase in the presence of oxygen, as required by applicants' claims.

It is respectfully requested that the foregoing amendments and remarks be taken into account prior to examination of the RCE application on the merits.

Respectfully submitted,

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